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# Serum Lipid Resistance to Oxidation and Uric Acid Levels in Subjects with Down's Syndrome

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## Summary

In subjects with Down's syndrome (DS) increased oxidative stress and consequent oxidative cell damage have been reported. The aim of this study was to assess whether the excessive production of free oxygen radicals in these subjects can affect the copper-induced lipid oxidation resistance measured in fresh whole serum. Since a significant elevation of serum uric acid levels, which is an efficient hydrophilic antioxidant, has been repeatedly reported in subjects with DS, we studied the association between increased serum uric acid levels and lipid resistance to oxidation measured directly in serum samples by monitoring the change in absorbance at 234 nm. The group of subjects with Down's syndrome consisted of 25 individuals (aged 18±5 years). Control group included brothers and sisters of subjects with DS (n = 25, aged 17±7 years). In subjects with DS, the serum lipid resistance to oxidation (lag time) was significantly higher than in controls ($p<0.05$) and a concomitant increase in serum uric acid levels was observed ($p<0.001$). A significant positive correlation between lag time and serum uric acid concentration was found in subjects with DS ($r = 0.48$, $p<0.05$), while the positive correlation in the control group was not significant. The results suggest that increased serum uric acid levels repeatedly observed in subjects with DS may be associated with an enhanced resistance of serum lipids to oxidation which is thought to play an important role in the atherogenic process.

**Key words**

Down's syndrome • Serum lipid resistance • Uric acid

## Introduction

Down's syndrome, or human trisomy 21, is the most frequent chromosomal abnormality which results from the presence of three copies of chromosome 21 instead of two. Due to this extra-chromosomal presence, many phenotypic and physiological factors are adversely affected. The overexpression of the enzyme superoxide dismutase (SOD), increased oxidative stress as a result of the excessive production of free oxygen radicals in all cells of patients with Down's syndrome and consequent oxidative cell damage have been reported (Sinet 1982). Down's syndrome is characterized by mental retardation, immunodeficiency, a wide variety of morphogenetic abnormalities including cardiomyopathy, gastrointestinal anomalies, increased incidence of leukemia, signs of premature aging and neuropathological alterations similar to those found in Alzheimer's disease (Pueschel and Pueschel 1992). In spite of these disorders, most of biochemical processes in persons with Down's syndrome are within normal limits. It also remains to be elucidated why the disturbances in lipid metabolism observed in subjects with DS do not increase their risk of atherosclerosis.



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Oxidation of serum lipoproteins, particularly the low density lipoproteins (LDL), is thought to play a major role in the development of atherosclerosis (Steinberg *et al.* 1989). The method of *in vitro* $Cu^{2+}$-mediated oxidation of isolated LDL is the most frequent method used to assess the oxidation resistance of LDL. The kinetics of conjugated diene formation during the oxidation of polyunsaturated fatty acids (PUFA) in the LDL is measured by monitoring the absorbance at 234 nm (Esterbauer *et al.* 1989). These measurements require time-consuming isolation of LDL which can result in partial oxidation of lipoprotein. Recently, several authors (Nyyssönen *et al.* 1997, Schnitzer *et al.* 1995, 1998, Spranger *et al.* 1998) have used the method of Regnström *et al.* (1993) to measure the lipid resistance to oxidation in whole plasma or serum. This is considered to represent a more adequate model of lipoprotein oxidation in the arterial wall than the *in vitro* oxidation of individual isolated lipoproteins (LDL, VLDL, HDL) where water-soluble antioxidants are artificially removed during the isolation. Unfractionated plasma or serum contains different hydrophilic constituents such as ascorbic acid, urate, bilirubin and albumin which protect plasma lipoproteins against oxidation *in vitro* and are also responsible for total peroxyl radical trapping capacity of the plasma (Wayner *et al.* 1987).

Urate is an efficient hydrophilic plasma antioxidant. Serum urate and ascorbate concentrations were the strongest determinants of serum lipid oxidation resistance, expressed as lag time, in a population sample of Finnish non-smokers (Nyyssönen *et al.* 1997). It was also hypothesized that uric acid might protect against cellular damage from free radicals in neurological diseases such as the Alzheimer type dementia or Parkinson's disease (Tohgi *et al.* 1993). The elevation of serum urate levels in subjects with Down's syndrome has been reported by several authors (Pueschel and Pueschel 1992), but the origin of this biochemical disturbance is not known. No previous studies have dealt with the possible antioxidant effect of increased serum uric acid levels on the oxidation of serum lipids in subjects with Down's syndrome.

The aim of this study was to find a relationship between genetically increased serum uric acid levels in subjects with Down's syndrome and the lipid resistance to oxidation as measured directly in serum samples by monitoring the change in absorbance at 234 nm.

## Materials and Methods

*Subjects and blood sampling*

The group of subjects with Down's syndrome consisted of 25 individuals (12 male and 13 female, mean age 18±5 years) selected from non-institutionalized persons, followed regularly in the Down's Syndrome Center at the Institute of Preventive and Clinical Medicine. Exclusion criteria for subjects with DS included abnormal thyroid values, congenital heart disease, diabetes mellitus, obesity or gastrointestinal disorders. The control group consisted of 25 healthy individuals (12 male and 13 female, mean age 17±7 years) who were brothers and sisters of the subjects with DS living in the same family. Informed consent was obtained from all the subjects and their parents and the study was approved by the Institute's Ethics Committee. Serum was isolated from blood samples collected after an overnight fast.

*Measurement of serum lipid resistance to oxidation*

The resistance of lipids to copper oxidation was measured in fresh serum according to Regnström *et al.* (1993). The serum was diluted a 150-fold in 0.02 mol/l phosphate buffered saline (PBS) pH=7.4. Oxidation was started by adding 1 mmol/l $CuCl_2$ into 2 ml of diluent serum (final concentration 50 μM) and the formation of conjugated dienes was measured by monitoring the change in 234 nm absorbance at 30 °C every 5 min for 5 h using a Beckman spectrophotometer DU-650 equipped with a six-position automatic sample changer. The lag time and the maximal rate of oxidation ($V_{max}$) were calculated from the oxidation curve as was previously described for LDL oxidation (Nagyová *et al.* 1998). $V_{max}$ was expressed as mabs/min.

*Other measurements*

Serum total cholesterol, HDL cholesterol and triglycerides were determined enzymatically (Boehringer, Mannheim, autoanalyzer Hitachi 911). LDL cholesterol was calculated from the Friedelwald formula and serum urates were determined using colorimetric, enzymatic method on the same autoanalyzer.

*Statistics*

Statistical evaluation was performed by using parametric Student's t-test for dependent samples and non-parametric Wilcoxon matched paired test to estimate differences between subjects with Down's syndrome and the control group. Associations between lipid resistance

parameters and other measurements were estimated by linear regression analysis. The threshold of statistical significance was $p<0.05$.

## Results and Discussion

Disturbances in lipid metabolism including a rise in LDL cholesterol and triglycerides, a decrease in HDL cholesterol as well as no changes in these lipid parameters in DS individuals were observed by several authors (Salo *et al.* 1979, Pueschel *et al.* 1992). We have found no differences in total and LDL serum cholesterol between subjects with DS and control group, although serum HDL cholesterol was significantly decreased and triglyceride levels were significantly enhanced in these subjects (Table 1). The same lipid profile in subjects with DS was observed by Pueschel *et al.* (1992), who concluded that the decreased prevalence of coronary artery disease observed by some authors in individuals with DS cannot be explained by this lipid profile.

**Table 1.** Serum lipid levels (mmol/l) in subjects with Down's syndrome and controls

| | Down's syndrome | Controls |
|---|---|---|
| *Total cholesterol* | 4.47±0.91 | 4.44±0.86 |
| *HDL cholesterol* | 1.29±0.36* | 1.57±0.40 |
| *LDL cholesterol* | 2.59±0.74 | 2.49±0.69 |
| *Triglycerides* | 1.23±0.70* | 0.84±0.39 |

*Values are means ± S.D. (n=25); * $p<0.05$.*

Serum lipid resistance to oxidation (lag time) was significantly higher in subjects with DS compared to the control group (160±42 vs. 138±33 s, $p<0.05$) (Fig. 1A). Besides this, the lag times in men of both studied groups were apparently longer than in women. We verified this observation independently in a group of healthy men (n=16) and women (n=18) where the lag time in men was also significantly longer than in women (unpublished results). The maximal rate of copper-induced serum lipid oxidation ($V_{max}$) was not changed in subjects with DS. The finding that persons with DS have a prolonged lag time compared to their healthy siblings is unexpected because of the excessive production of free oxygen radicals characteristic for these subjects and the following increase of plasma lipoperoxides, measured as thiobarbituric acid reactive substances (Kędziora *et al.* 1986). Many different factors may contribute to the observed increased resistance of serum lipids to oxidation. The increased activities of protective antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), which are accompanied by increased oxidative stress in subjects with DS, disturbances in lipid metabolism and lipid composition or changes in serum total antioxidant capacity, all may affect serum lipid oxidation. Moreover, other factors specific for this genetic disease, which is accompanied by several endocrinological, hematological, metabolic and immunological disturbances, may also be important in this respect.

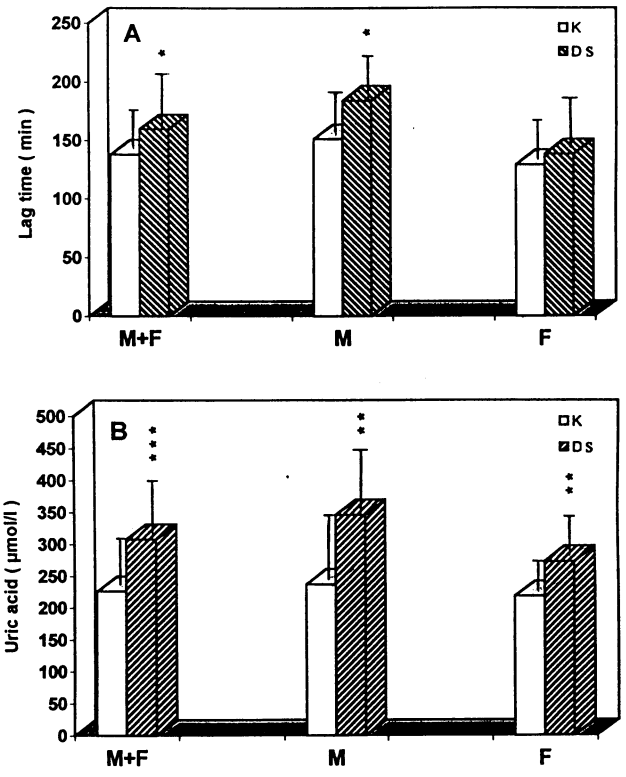


**Fig. 1.** *[A] Serum lipid resistance to oxidation (lag time) in subjects with Down's syndrome (DS) and controls (K). [B] Uric acid levels in serum from subjects with Down's syndrome and controls. Values are means ± S.D. Significant differences from controls: * $p<0.05$; ** $p<0.01$; *** $p<0.001$. M + F – male (n=12) + female (n=13). M – male (n=12). F – female (n=13).*

Our results confirmed the increased serum uric acid levels reported for subjects with DS by other authors (Fig. 1B). A significant increase of serum uric acid in subjects with DS was more evident in men (increased by 46 %, $p<0.01$) than in women (increased by 25 %, $p<0.01$). We found a significant positive correlation between serum lipid resistance to oxidation (lag time) and serum uric acid levels in subjects with DS ($r = 0.48$, $p<0.05$), but in control individuals this positive correlation was not significant. There were also strong positive correlations in subjects with DS between serum uric acid levels and serum total cholesterol ($r = 0.57$, $p<0.01$), LDL cholesterol ($r = 0.57$, $p<0.01$) and triglycerides ($r = 0.52$, $p<0.01$), while in control individuals uric acid correlated significantly with triglycerides only ($r = 0.44$, $p<0.05$). It should be noted that almost all serum uric acid and serum lipid values were within the range of physiological values. Hyperuricemia is often associated with dyslipidemia, obesity, hypertension or diabetes, which are thought to be risk factors for cardiovascular diseases. A positive association between serum triglycerides and uric acid levels were observed in healthy men as well as in the insulin resistance syndrome. Elevated triglyceride levels seem to be independent of hyperuricemia (Bonora *et al.* 1996, Rathmann *et al.* 1998). Uric acid is an efficient hydrophilic plasma antioxidant, which acts as a radical scavenger and also inhibits the iron-catalyzed oxidation of ascorbate (Sevanian *et al.* 1991). According to Wayner *et al.* (1987), uric acid contributed to plasma total peroxyl radical trapping capacity in the range from 35 % to 65 %. The other main contributors were plasma ascorbic acid, -SH groups and vitamin E. Ascorbate and urate were also the strongest determinants of plasma antioxidative capacity and serum lipid resistance to oxidation in Finnish non-smokers (Nyyssönen *et al.* 1997). Recently, serum antioxidant status was determined in persons with DS and their siblings (control group) (Ďuračková *et al.* 1997). The subjects with DS had significantly higher uric acid and ascorbate levels (by 50 % and 20 %, respectively), but vitamin E concentration was increased non-significantly (by 8 %). With regard to the fact that many subjects enrolled in the study of Ďuračková *et al.* (1997) also participated in our study, we could suppose that our subjects with DS also had higher serum ascorbate levels. This would be in agreement with the protective antioxidative effect of urate which saves ascorbic acid in biological systems.

Our results suggest that increased serum uric acid levels repeatedly observed in DS subjects may be associated with the increased resistance of serum lipids to oxidation which is thought to play an important role in the atherogenic process.

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